MycoKeys 106: 287–301 (2024)
DOI: 10.3897/mycokeys.106.117205

Research Article

Multi-locus molecular phylogenetic analysis reveals two new species of *Amphichorda* (Bionectriaceae, Hypocreales)

Zhi-Qin Wang[1,2*], Jing Zhao[1,2*], Quan-Ying Dong[1,2], Yao Wang[1,2], Ying-Ling Lu[1,2], Run Luo[1,2], Hong Yu[1,2]

1 *Yunnan Herbal Laboratory, College of Ecology and Environmental Sciences, Yunnan University, Kunming, Yunnan, China*
2 *The International Joint Research Center for Sustainable Utilization of Cordyceps Bioresources in China and Southeast Asia, Yunnan University, Kunming, Yunnan, China*

Corresponding author: Hong Yu (hongyu@ynu.edu.cn, herbfish@163.com)

Abstract

Amphichorda has been previously accepted as a member of the Cordycipitaceae and currently it is considered a member of the Bionectriaceae. The substrates of *Amphichorda* were complex and varied, being mainly animal faeces. This study reports two new species of *Amphichorda* from Yunnan Province in south-western China. Based on the five-gene (nr*SSU*, nr*LSU*, *tef-1α*, *rpb1* and *rpb2*) sequence and ITS data phylogenetic analysis, two new species, namely *A. excrementa* and *A. kunmingensis*, are proposed and a detailed description of the new species is provided. *Amphichorda excrementa* and *A. kunmingensis* were isolated from animal faeces in the park. The morphological characteristics of two novel species and seven known species in *Amphichorda* are also compared.

Key words: Coprophilous fungi, diversity, morphology, new taxa, taxonomy

OPEN ACCESS

Academic editor: Nalin Wijayawardene
Received: 11 December 2023
Accepted: 6 May 2024
Published: 3 July 2024

Citation: Wang Z-Q, Zhao J, Dong Q-Y, Wang Y, Lu Y-L, Luo R, Yu H (2024) Multi-locus molecular phylogenetic analysis reveals two new species of *Amphichorda* (Bionectriaceae, Hypocreales). MycoKeys 106: 287–301. https://doi.org/10.3897/mycokeys.106.117205

Copyright: © Zhi-Qin Wang et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

Amphichorda Fr. was established to accommodate the type species *A. felina* (DC.) Fr., which was isolated from cat dung and previously classified in the genus *Clavaria* (Lamarck 1815; Fries 1825). At the present, seven species of the *Amphichorda* are now validly published (Zhang et al. 2017, 2021; Guerra-Mateo et al. 2023; Liu et al. 2023; Leão et al. 2024). The traditional phylogenetic placement of the genus *Amphichorda* was considered in the family Cordycipitaceae (Hypocreales). The Cordycipitaceae is the most complex group in the order Hypocreales because of its varied morphological characteristics and wide-ranging hosts and some genera present numerous taxonomical problems (Wang et al. 2020; Guerra-Mateo et al. 2023). In the studies of Zhang et al. (2017, 2021) and Liu et al. (2023) which report new species of the genus *Amphichorda*, the phylogenetic position of *Amphichorda* belongs to the Cordycipitaceae. However, Guerra-Mateo et al. (2023) conducted the phylogenetic analysis based on the nuclear ribosomal internal transcribed spacer region (ITS) and the nuclear ribosomal large subunit (nr*LSU*), considered *Amphichorda* to belong to the family Bionectriaceae and determined *Amphichorda* has close phylogenetic

* These authors contributed equally to this work.

relationships with the genera *Hapsidospora* and *Nigrosabulum*. Leão et al. (2024) also proving the genus *Amphichorda* belongs to the family Bionectriaceae.

The taxonomic status of the type species has been controversial since the original description of the type species of the *Amphichorda*. *Amphichorda felina* was classified as *Beauveria* in 1980 (Carmichael et al. 1980). However, early phylogenetic analyses showed that *Beauveria felina* was distant from other *Beauveria* species and that it was morphologically distinguished from other *Beauveria* species by the absence of elongate conidiogenous cells with apical denticulate rachis (Rehner et al. 2011; Zhang et al. 2017; Liu et al. 2023). The type strain of *A. felina* (= *B. felina*) seems to be unknown (Guerra-Mateo et al. 2023). *Isaria cretacea* J.F.H. Beyma type strain CBS 250.34 was considered to be the type strain of *A. felina* since *I. cretacea* was synonymised with *A. felina* (De Hoog 1972; Zhang et al. 2021; Guerra-Mateo et al. 2023). However, the criteria required for fungal epitypification were the substrate and geographic similarity (Guerra-Mateo et al. 2023). The substrate and geography were different between *A. felina* and the strain CBS 250.34, so this strain has not been designated as the epitype of *A. felina* (Lendemer 2020). Guerra-Mateo et al. (2023) proposed that the strain CBS 250.34 can be accepted as a reference to stabilise the nomenclature of *A. felina*, but should be avoided to indicate it as a type strain of *A. felina* (Zhang et al. 2017, 2021; Wang et al. 2020; Liu et al. 2023).

During the surveys of entomopathogenic fungi from two regions in Yunnan Province, China, the animal faeces were collected and three strains were isolated from the specimens. Based on morphological evidence together with the five-gene (nr*SSU*, nr*LSU*, *tef-1α*, *rpb1* and *rpb2*) sequence and ITS data analyses of some genera in Bionectriaceae, it was shown that the three strains belong to the genus *Amphichorda*. On the basis of its morphological characteristics and multi-locus molecular phylogenetic analyses, two new species were described. Furthermore, the morphological characteristics of two novel species and seven known species in *Amphichorda* were compared.

Materials and methods

Fungal collection and isolation

The specimens were collected in Kunming City, Yunnan Province, China in July 2019. In the field, it was placed in sterilised plastic pipes and brought to the laboratory for isolation. In order to obtain axenic cultures, part of the surface tissue of the specimen was cut off with a sterilised dissecting knife and then placed into a flask containing 10 ml of sterilised water and glass beads. Then the suspension was shaken for 10 min and diluted 50 times. Finally, the diluted suspension was applied on Petri dishes with potato dextrose agar (PDA: fresh potato 200 g/l, dextrose 20 g/l and agar 18 g/l) containing 0.1 g/l streptomycin and 0.05 g/l tetracycline. Then the Petri dish was placed in a room at 15 °C to allow it to grow, during which time the growing fungi were transferred one by one to new Petri dishes. After isolation into pure cultures, they were transplanted to a PDA slant and stored at 4 °C. The specimens were deposited in the Yunnan Herbal Herbarium (YHH) of Yunnan University, China. The strain was deposited at the Yunnan Fungal Culture Collection (YFCC) of Yunnan University, China. The culture of the *Amphichorda felina* (CBS 250.34) was obtained from

the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute (WI) in Utrecht, the Netherlands. The obtained strain CBS 250.34 was inoculated into PDA medium and re-cultured.

Morphological observations

Colonies were incubated on PDA for three weeks in an incubator at 25 °C. The photograph was taken morphologically using a Canon 750 D camera (Canon Inc., Tokyo, Japan). The anamorphs (Conidiophores, Phialides and Conidia) in culture were observed using a light microscope (Olympus BX53). The growth rate of colonies was calculated using the method of Liu and Hodge (2005) and it was categorised as: fast-growing (30–35 mm in diameter), moderately growing (20–30 mm in diameter) and slow-growing (< 20 mm in diameter).

DNA extraction, PCR and sequencing

The genomic DNA was extracted from axenic living cultures using the Genomic DNA Purification Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The five-gene (nr*SSU*, nr*LSU*, *tef-1α*, *rpb1* and *rpb2*) and ITS were sequenced and the following primer pairs were used for PCR amplification. The nuclear ribosomal internal transcribed spacer region (ITS) was amplified with the primer pairs ITS4/ITS5 (White et al. 1990). The nuclear ribosomal small and large subunit (nr*SSU* and nr*LSU*) were amplified with the primer pairs nr*SSU*-CoF/nr*SSU*-CoR and LR5/LR0R, respectively (Vilgalys and Hester 1990; Rehner and Samuels 1994; Wang et al. 2015a). The translation elongation factor 1α (*tef-1α*) was amplified with the primers EF1α-EF and EF1α-ER (Bischof et al. 2006; Sung et al. 2007). The largest and second subunits of RNA polymerase II (*rpb1* and *rpb2*) were amplified with the primers RPB1-5'F/RPB1-5'R and RPB2-5'F/RPB2-5'R, respectively (Bischof et al. 2006; Sung et al. 2007). The polymerase chain reaction (PCR) matrix was performed in a final volume of 50 µl and the detailed information was described by Wang et al. (2022). Amplification reactions were performed in the BIORAD T100TM thermal cycler (BIO-RAD Laboratories, Hercules, CA, United States). The PCR reactions followed the procedures of Wang et al. (2015b) and the PCR products were sequenced by the Beijing Genomics Institute (Chongqing, China).

Phylogenetic analyses

Based on the six-locus molecular, including ITS, nr*SSU*, nr*LSU*, *tef-1α*, *rpb1* and *rpb2*, phylogenetic analyses were performed using datasets retrieved from GenBank and those generated in this work. The DNA sequences newly generated have been submitted to GenBank. The sequences downloaded from the GenBank database were based on a previous study by Hou et al. (2023) and Leão et al. (2024). The taxonomic information and corresponding GenBank accession numbers used are provided in Table 1. Sequences were aligned with MEGA v.6.06 and used to remove poorly-aligned regions and for manual adjustment (Tamura et al. 2013). Six-locus molecular were concatenated together using Phylosuite v.1.2.2 (Zhang et al. 2020). The Maximum Likelihood (ML) tree was performed using IQ-tree v.2.1.3 and the Bayesian Inference (BI) tree was performed using MrBayes v.3.2.2

Table 1. Species information and corresponding GenBank accession numbers of *Amphichorda* and close relative genera used in this study.

Species	Strain	ITS	nr*SSU*	nr*LSU*	*tef 1α*	*rpb1*	*rpb2*
Alloacremonium humicola	CBS 613.82	NR_189433	–	NG_229089	OQ470786	–	OQ453888
Alloacremonium ferrugineum	CBS 102877	NR_189432	–	NG_228721	OQ470785	–	OQ453887
*Amphichorda cavernicol*a	CGMCC3.19571	MK329056	–	MK328961	MK335997	–	–
Amphichorda cavernicola	LC12481	MK329057	–	MK328962	MK335998	–	–
Amphichorda cavernicola	LC12553	MK329059	–	MK328964	MK336000	–	–
Amphichorda cavernicola	LC12560	MK329061	–	MK328966	MK336002	–	–
Amphichorda coprophila	CBS 247.82 ^{T}	MH861494	–	MH873238	OQ954487	–	–
Amphichorda coprophila	CBS 424.88	OQ942929	–	OQ943166	OQ954488	–	–
Amphichorda excrementa	**YFCC AECCS848^{T}**	-	**OR913433**	**OR913439**	**OR917446**	**OR917451**	**OR917443**
Amphichorda felina	CBS 250.34	MH855498	–	OQ943167	OQ954490	–	–
Amphichorda felina	**CBS 250.34**	-	**OR913436**	**OR913440**	**OR917447**	**OR917450**	**OR917444**
Amphichorda felina	CBS 648.66	OQ942930	–	MH870575	OQ954491	–	–
Amphichorda guana	CGMCC3.17908^{T}	KU746665	KY883262	KU746711	KX855211	KY883202	KY883228
Amphichorda guana	CGMCC3.17909	KU746666	KY883263	KU746712	KX855212	KY883203	–
Amphichorda kunmingensis	**YFCC AKYYH8414^{T}**	-	**OR913435**	**OR913438**	**OR917448**	**OR917452**	–
Amphichorda kunmingensis	**YFCC AKYYH8487**	-	**OR913434**	**OR913437**	**OR917449**	**OR917453**	**OR917445**
Amphichorda littoralis	FMR 17952	OQ942925	–	OQ943162	OQ954483	–	–
Amphichorda littoralis	FMR 19404^{T}	OQ942924	–	OQ943161	OQ954482	–	–
Amphichorda littoralis	FMR 19611	OQ942926	–	OQ943163	OQ954484	–	–
Amphichorda monjolensis	COAD 3124	OQ288256	–	OQ288260	OR454090	–	OQ405040
Amphichorda monjolensis	COAD 3125	OQ288257	–	–	–	–	OQ405041
Amphichorda monjolensis	COAD 3120	OQ288258	–	–	–	–	OQ405042
Amphichorda yunnanensis	KUMCC 21-0414	ON426823	–	–	OR025977	OR022016	OR022041
Amphichorda yunnanensis	KUMCC 21-0415	ON426824	–	–	OR025976	OR022015	OR022040
Amphichorda yunnanensis	KUMCC 21-0416^{T}	-	–	–	OR025975	OR022014	OR022039
Bulbithecium ammophilae	CBS 178.78	NR_189437	–	NG_242039	OQ470793	–	OQ453895
Bulbithecium arxii	CBS 737.84	NR_145040	–	HQ232159	OQ470794	–	OQ451834
Bulbithecium borodinense	CBS 101148	OQ429506	–	HQ232003	–	–	–
Bulbithecium ellipsoideum	CBS 993.69	NR_189438	–	NG_242040	OQ470796	–	OQ453896
Bulbithecium hyalosporum	CBS:318.91	MH862256	AF096172	OQ055419	OQ470797	–	OQ453897
Bulbithecium pinkertoniae	CBS 157.70	NR_159611	NG_062816	NG_058554	OQ470799	–	OQ453898
Bulbithecium spinosum	CBS 136.33	OQ429512	NG_062819	NG_056971	OQ470802	–	OQ453899
Bulbithecium truncatum	CBS 113718	NR_189439	–	NG_242041	OQ470803	–	OQ453900
Claviceps purpurea	SA cp11	-	EF469122	EF469075	EF469058	EF469087	EF469105
Geosmithia lavendula	CBS 344.48	MH856380	–	MH867927	–	–	–
Geosmithia pallidum	CBS 260.33	OQ429599	–	OQ055509	OQ470909	–	OQ453998
Hapsidospora chrysogena	CBS 144.62	NR_189452	NG_062810	HQ232017	OQ470953	–	OQ454043
Hapsidospora flava	CBS 596.70	NR_189453	NG_062812	NG_056983	OQ470957	–	OQ454047
Hapsidospora globosa	CBS 512.70	NR_160124	–	NG_064081	OQ470963	–	OQ454053
Hapsidospora inversa	CBS 517.70	NR_189454	–	OQ055565	OQ470967	–	OQ454057
Hapsidospora irregularis	CBS 510.70	NR_160123	–	MH871595	OQ470968	–	OQ454058
Hapsidospora stercoraria	CBS 516.70	OQ429662	–	OQ055568	OQ470970	–	OQ454060
Hapsidospora variabilis	CBS 100549	NR_189456	–	NG_229091	OQ470971	–	OQ454061
Myriogenospora atramentosa	AEG 96-32	-	AY489701	AY489733	AY489628	AY489665	DQ522455

Species	Strain	ITS	nr*SSU*	nr*LSU*	*tef 1α*	*rpb1*	*rpb2*
Ovicillium subglobosum	CBS 101963	NR_154335	–	NG_069329	OQ471085	–	OQ454170
Ovicillium attenuatum	CBS 399.86	NR_154333	–	NG_229092	OQ471083	–	OQ454168
Proxiovicillium blochii	CBS 427.93	-	HQ232182	HQ232001	–	–	–
Proxiovicillium lepidopterorum	CBS 101239	NR_189482	–	NG_242070	OQ471145		OQ454214
Proliferophialis apiculata	CBS 303.64	NR_189480	–	NG_242064	OQ471122	–	OQ454207
Proliferophialis apiculata	CBS 397.78	OQ429798	–	OQ055694	–	–	OQ454209
Stilbocrea macrostoma	CBS 141849	OQ429874	–	OQ430123	–	–	OQ454273
Stilbocrea walteri	CBS 144627	NR_160063	–	NG_242075	–	–	–
Waltergamsia parva	CBS 381.70A	NR_163808	–	NG_242083	OQ471279	–	OQ454346
Waltergamsia pilosa	CBS 124.70	NR_163809	–	OQ430199	OQ471282	–	OQ454349

Boldface: data generated in this study; [T]: ex-type culture.
A.E.G: A. E. Glenn personal collection; CBS: the culture collection of the Westerdijk Fungal Biodiversity Institute (WI); CGMCC: the China General Microbiological Culture Collection Center; COAD: the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas and Coleção Octávio Almeida Drummond; FMR: the culture collection of the Faculty of Medicine in Reus; KUMCC: the Kunming Culture Collection; LC: personal culture collection held in the lab of Dr Lei Cai; YFCC: the Yunnan Fungal Culture Collection (YFCC) of Yunnan University.

(Ronquist et al. 2012; Nguyen et al. 2015). The best-fitting likelihood model for BI and ML analyses was selected using ModelFinder (Kalyaanamoorthy et al. 2017). In the phylogenetic tree of *Amphichorda* and some other genera, the TN+F+I+G4 model was selected as the optimal model for the ML analyses, with 5000 ultrafast bootstraps (Hoang et al. 2017) in a single run. The GTR+F+I+G4 model was selected as the optimal model for the BI analysis and the four Markov Chain Monte Carlo chains run for 2 million generations from a random start tree with a sampling frequency of 100 generations, in which the initial 25% of sampled data were discarded as burn-in. Phylogenetic trees were visualised in FigTree v.1.4.3 and edited in Adobe Illustrator CS6. The values of ML bootstrap proportions (BP) (≥ 70%) and the BI posterior probability (PP) (≥ 0.70) are indicated at the nodes (BP/PP).

Results

Sequencing and phylogenetic analyses

The phylogenetic tree was inferred using 54 strains of 12 genera from Bionectriaceae and Clavicipitaceae, including *Alloacremonium*, *Amphichorda*, *Bulbithecium*, *Claviceps*, *Geosmithia*, *Hapsidospora*, *Myriogenospora*, *Ovicillium*, *Proxiovicillium*, *Proliferophialis*, *Stilbocrea* and *Waltergamsia*. Two strains (*Claviceps purpurea* SA cp11 and *Myriogenospora atramentosa* AEG 96-32) of Clavicipitaceae were selected as the outgroup. The final length of the six-locus molecular sequence concatenated dataset was 5,798 bp, including 766 bp for ITS, 1,391 bp for nr*SSU*, 859 bp for nr*LSU*, 850 bp for *tef-1α*, 781 bp for *rpb1* and 1,151 bp for *rpb2*. Phylogenetic trees from the BI and ML analyses exhibited similar topologies that had ten recognised, statistically well-supported clades in Bionectriaceae. The four strains were clustered in the genus *Amphichorda* based on the phylogenetic analyses of the combined dataset (Fig. 1). Our ML and BI analyses showed that two new species (i.e. *A. excrementa* and *A. kunmingensis*) and one known species were recognised. The new species, *A. excrementa* and *A. kunmingensis*, were well-supported by bootstrap proportions (BP = 90% and BP = 82%, respectively) and posterior probabilities (PP = 1.00 and PP = 0.96, respectively).

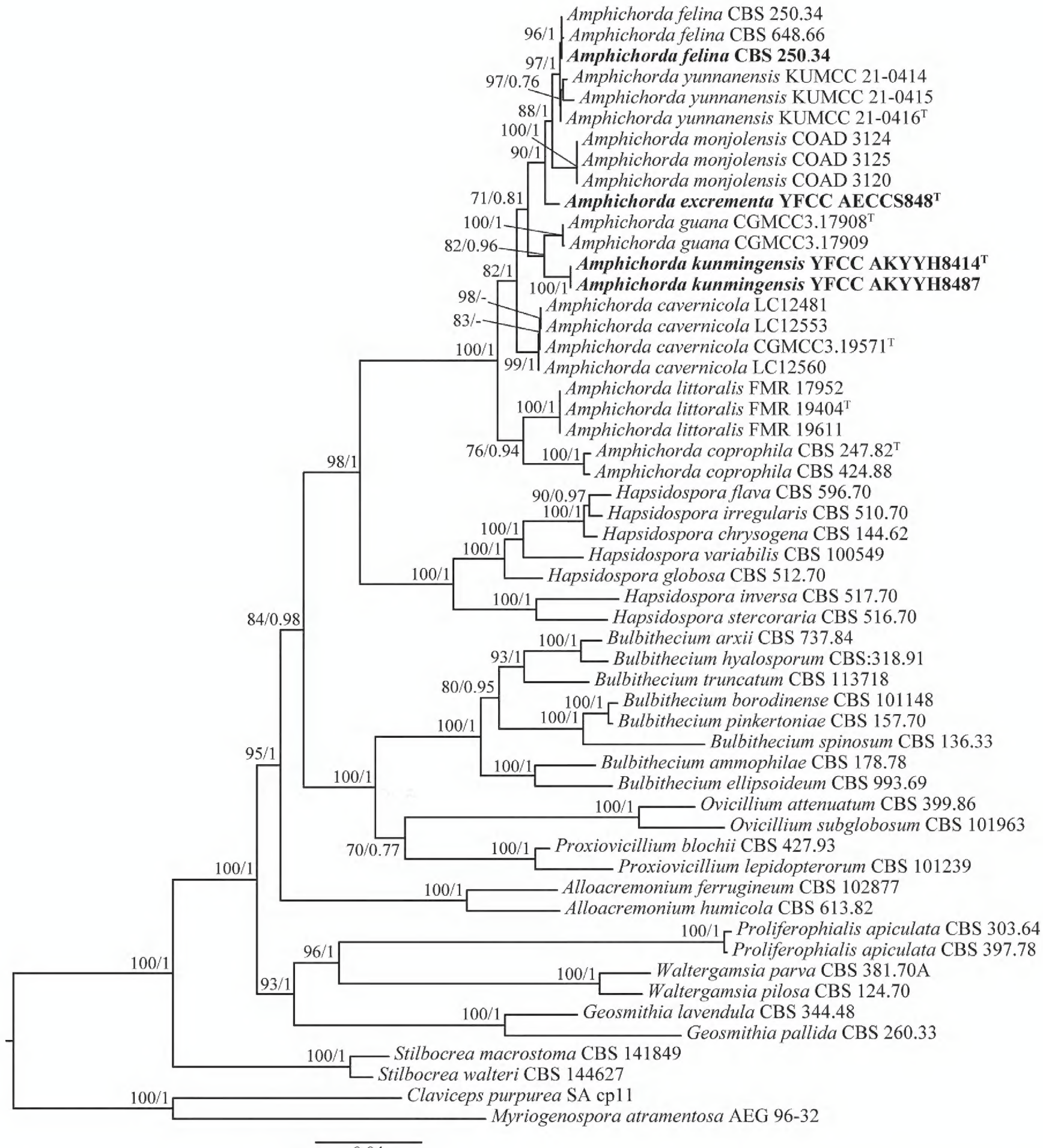
Amphichorda felina CBS 250.34
96/1 Amphichorda felina CBS 648.66
Amphichorda felina CBS 250.34
97/1 Amphichorda yunnanensis KUMCC 21-0414
97/0.76 Amphichorda yunnanensis KUMCC 21-0415
88/1 Amphichorda yunnanensis KUMCC 21-0416T
100/1 Amphichorda monjolensis COAD 3124
90/1 Amphichorda monjolensis COAD 3125
Amphichorda monjolensis COAD 3120
71/0.81 Amphichorda excrementa YFCC AECCS848T
100/1 Amphichorda guana CGMCC3.17908T
82/0.96 Amphichorda guana CGMCC3.17909
Amphichorda kunmingensis YFCC AKYYH8414T
82/1 Amphichorda kunmingensis YFCC AKYYH8487
100/1 Amphichorda cavernicola LC12481
98/- Amphichorda cavernicola LC12553
83/- Amphichorda cavernicola CGMCC3.19571T
100/1 Amphichorda cavernicola LC12560
99/1 Amphichorda littoralis FMR 17952
Amphichorda littoralis FMR 19404T
100/1 Amphichorda littoralis FMR 19611
76/0.94 Amphichorda coprophila CBS 247.82T
100/1 Amphichorda coprophila CBS 424.88
98/1 Hapsidospora flava CBS 596.70
90/0.97 Hapsidospora irregularis CBS 510.70
100/1 Hapsidospora chrysogena CBS 144.62
100/1 Hapsidospora variabilis CBS 100549
100/1 Hapsidospora globosa CBS 512.70
100/1 Hapsidospora inversa CBS 517.70
100/1 Hapsidospora stercoraria CBS 516.70
84/0.98 Bulbithecium arxii CBS 737.84
100/1 Bulbithecium hyalosporum CBS:318.91
93/1 Bulbithecium truncatum CBS 113718
80/0.95 Bulbithecium borodinense CBS 101148
100/1 Bulbithecium pinkertoniae CBS 157.70
100/1 Bulbithecium spinosum CBS 136.33
95/1 Bulbithecium ammophilae CBS 178.78
100/1 Bulbithecium ellipsoideum CBS 993.69
100/1 Ovicillium attenuatum CBS 399.86
Ovicillium subglobosum CBS 101963
70/0.77 Proxiovicillium blochii CBS 427.93
100/1 Proxiovicillium lepidopterorum CBS 101239
100/1 Alloacremonium ferrugineum CBS 102877
Alloacremonium humicola CBS 613.82
100/1 Proliferophialis apiculata CBS 303.64
96/1 Proliferophialis apiculata CBS 397.78
100/1 Waltergamsia parva CBS 381.70A
100/1 93/1 Waltergamsia pilosa CBS 124.70
100/1 Geosmithia lavendula CBS 344.48
Geosmithia pallida CBS 260.33
100/1 Stilbocrea macrostoma CBS 141849
Stilbocrea walteri CBS 144627
100/1 Claviceps purpurea SA cp11
Myriogenospora atramentosa AEG 96-32
0.04

Figure 1. Phylogenetic tree of *Amphichorda* and close relative genera was constructed, based on Maximum Likelihood (ML) and Bayesian Inference (BI) analysis using six-locus molecular (ITS, nr*SSU*, nr*LSU*, *tef-1α*, *rpb1* and *rpb2*) sequences. The values of ML bootstrap proportions (BP) (≥ 70%) and the BI posterior probability (PP) (≥ 0.70) are indicated at the nodes (BP/PP). The new taxa were highlighted in bold.

Taxonomy

Amphichorda excrementa **Hong Yu bis, Z.Q. Wang, Q.Y. Dong & Y. Wang, sp. nov.**

MycoBank No: 851377

Fig. 2

Etymology. Refers to the excrement material from which this fungus was isolated.

Type. China, Yunnan Province, Kunming City, Changchongshan Country Park, 11 July 2019, Hong Yu and Yao Wang (YHH AECCS200777, ***holotype***; YFCC AECCS848, ex-type).

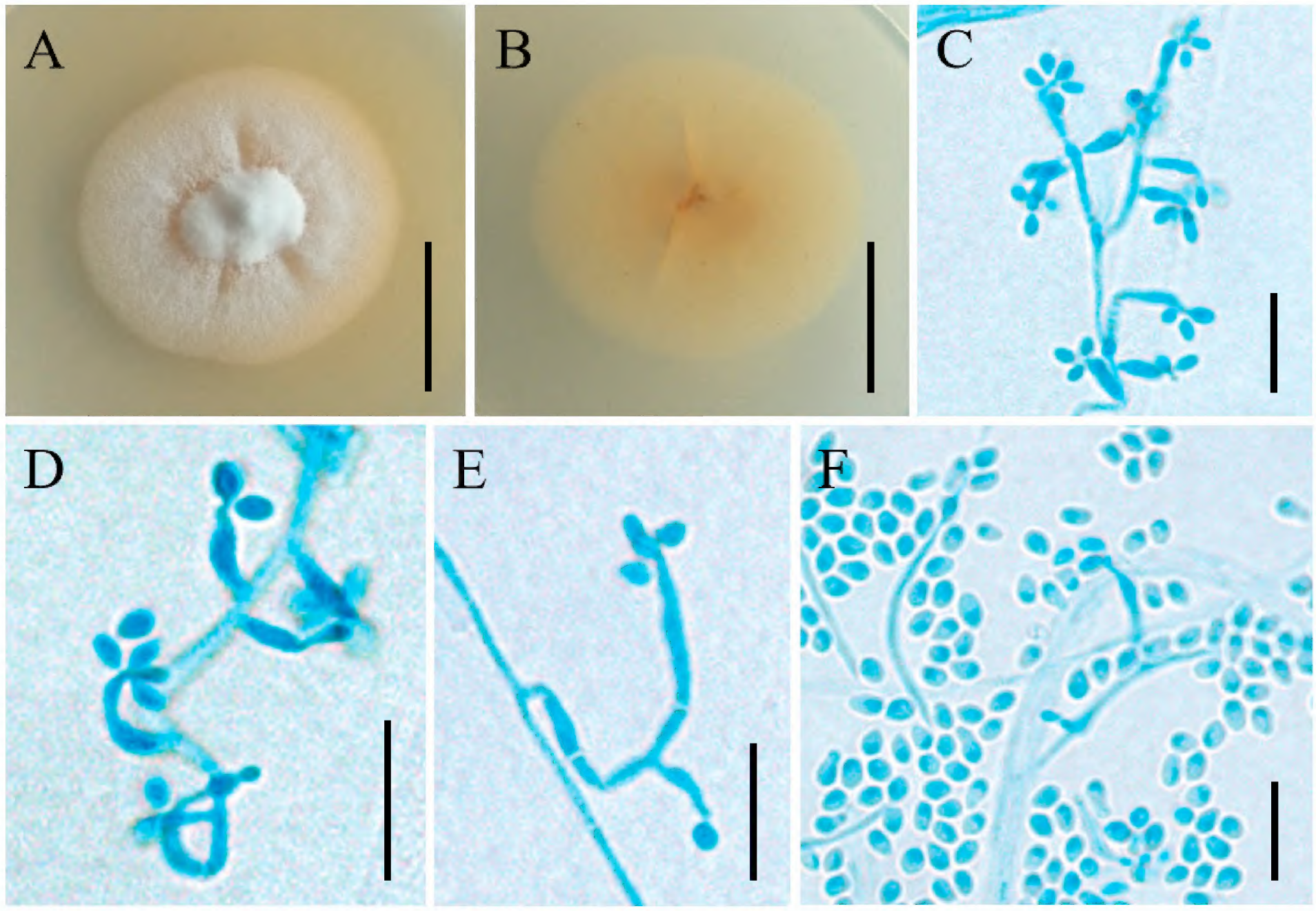

Figure 2. Morphology of *Amphichorda excrementa* **A, B** colony character on PDA medium after 30 d (**A** obverse **B** reverse) **C–F** conidiophores, conidiogenous cells and conidia. Scale bars: 2 cm (**A, B**); 10 μm (**C–F**).

Description. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Colonies on PDA attaining a diameter of 42–44 mm after a month at 25 °C, white to cream, with high mycelial density, cottony, with a yellow margin, reverse pale yellow. ***Hyphae*** branched, smooth-walled, septate, hyaline, 0.6–1.3 μm wide. Cultures readily produced phialides and conidia after 3 weeks on potato dextrose agar at room temperature. ***Conidiophores*** arising laterally from hyphae, cylindrical, straight or slightly curved, hyaline and occasionally branched. ***Phialides*** arising laterally from aerial hyphae, occasionally solitary, mostly in whorls of 2–3 on lateral branches from the mycelia, basal portion cylindrical or flask-shaped, usually curved, 4.1–13.9 × 1.3–2.1 μm, tapering abruptly towards the apex, have a distinctly thin neck. ***Conidia*** 1.7–3.0 × 1.2–2.5 μm, one-celled, smooth-walled, hyaline, globose to elliptical, single. ***Chlamydospores*** not observed.

Substrate. Animal faeces.

Distribution. China.

Commentary. Phylogenetic analyses showed that *Amphichorda excrementa* formed a separate clade with statistical support from the BI posterior probabilities (PP = 1.00) and the ML bootstrap proportions (BP = 90%) and was closely related to *A. felina*, *A. yunnanensis* and *A. monjolensis*. However, *A. excrementa* can be distinguished from three species by morphological differences. The phialides of *A. excrementa* were longer (4.1–13.9 × 1.3–2.1 μm) than those of *A. felina* (1.5–8.5 × 1.8–2.9 μm) and the conidia were smaller than those of *A. felina* (1.7–3.0 × 1.2–2.5 μm vs. 2.5–4.7 × 2–3.5 μm). The phialides of

A. excrementa were longer (4.1–13.9 × 1.3–2.1 µm) than those of *A. yunnanensis* (4–12 × 1–4 µm) and the conidia were smaller than those of *A. felina* (1.7–3.0 × 1.2–2.5 µm vs. 2–5 × 2–4 µm). The conidia of *A. monjolensis* were longer than those of *A. excrementa* (2.8–3.7 × 1.8–2.9 µm vs. 1.7–3.0 × 1.2–2.5 µm).

***Amphichorda felina* (DC.) Fr., Syst. orb. veg. (Lundae) 1: 170 (1825).**

MycoBank No: 562082

Fig. 3

Description. The morphological description of this study is based on the specimen, CBS 250.34. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Colonies on PDA attaining a diameter of 36–38 mm after a month at 25 °C, white to creamy-white, hard texture, felt-like, reverse black-brown, many conidia assemble to form powder. ***Hyphae*** branched, smooth-walled, septate, hyaline, 1.2–2.4 µm wide. ***Phialides*** arising laterally from aerial hyphae, erect or irregularly curved, 1.5–4.1 × 1.8–2.9 µm. ***Conidia*** 2.9–4.7 × 2.4–3.5 µm, one-celled, smooth-walled, hyaline, broadly ellipsoid or subglobose, single or aggregated into spheres. ***Chlamydospores*** not observed.

Substrate. Pupa of *Anaitis efformata*, rabbit dung, mouldy leaves, porcupine dung, cat dung.

Distribution. Argentina, Britain, France, Germany.

Commentary. Guerra-Mateo et al. (2023) proposed that the strain CBS 250.34 can be accepted as a reference to stabilise the nomenclature of *Amphichorda felina* and thus the genus *Amphichorda*, but should be avoided to indicate it as a type strain. In this study, the strain, CBS 250.34, was available in the CBS culture collection and morphological observations were made. Its morphology was generally consistent with those described by De Hoog (1972), with one difference being that this study extended the phialides (1.5–8.5 × 1.8–2.9 µm) and conidia size range of this species (2.5–4.7 × 2–3.5 µm).

***Amphichorda kunmingensis* Hong Yu bis, Z.Q. Wang, Q.Y. Dong & Y. Wang, sp. nov.**

MycoBank No: 851378

Fig. 4

Etymology. Named from the location Kunming City where the species was collected.

Type. China, Yunnan Province, Kunming City, Wild Duck Lake Forest Park, 16 July 2019, Hong Yu and Yao Wang (YHH AKYYH200704, ***holotype***; YFCC AKYYH8414, ex-type).

Description. *Sexual morph*: Undetermined. ***Asexual morph*:** Colonies on PDA attaining a diameter of 52–54 mm after a month at 25 °C, white to pale grey, with low mycelial density, lanose. ***Hyphae*** hyaline, branched, smooth-walled, septate, 0.7–1.9 µm wide. Cultures readily produced phialides and conidia after 3 weeks on potato dextrose agar at room temperature. ***Phialides*** arising laterally from aerial hyphae, solitary, occasionally in simple whorls on lateral branches from the mycelia, basal portion cylindrical or fusiform, straight or

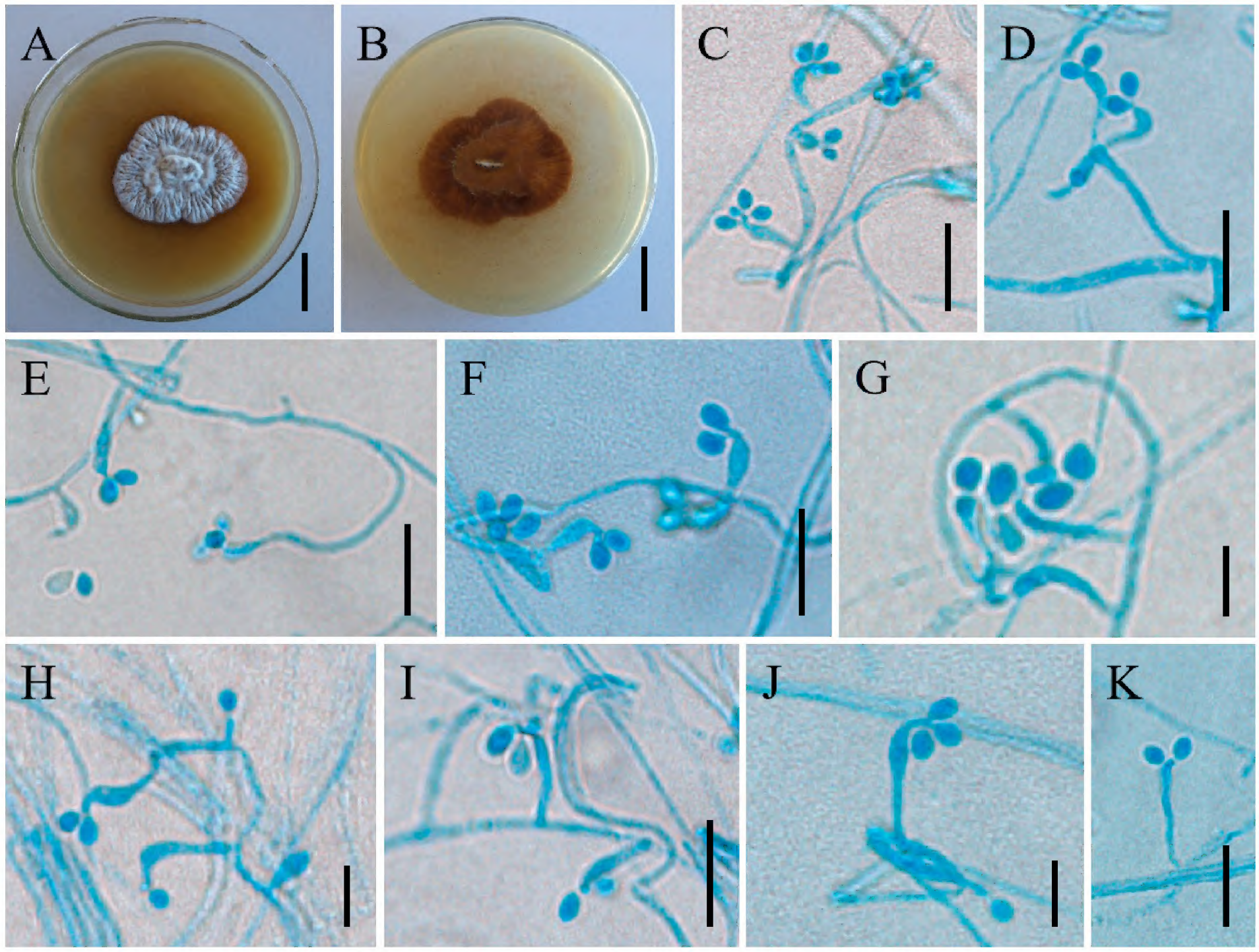

Figure 3. Morphology of *Amphichorda felina* **A, B** colony character on PDA medium after 30 d (**A** obverse **B** reverse) **C–K** conidiophores, conidiogenous cells and conidia. Scale bars: 2 cm (**A, B**); 10 μm (**C–F, I, K**); 5 μm (**G–H, J**).

irregularly bent, 6.1–17.5 × 1.4–2.9 μm. ***Conidia*** 2.3–4.2 × 1.6–3.0 μm, one-celled, smooth-walled, hyaline, globose to elliptical, single or aggregating in small heads at the apex of conidiogenous cells. ***Chlamydospores*** not observed.

Substrate. Animal faeces.

Distribution. China.

Other material examined. CHINA, Yunnan Province, Kunming City, Wild Duck Lake Forest Park, 16 July 2019, Hong Yu and Yao Wang (YHH AKYYH200776, paratype; YFCC AKYYH8487, ex-paratype).

Commentary. Three species of *Amphichorda* were from China and *A. yunnanensis* was distributed in Yuxi City, Yunnan Province. The two new species in this study were from Kunming City, Yunnan Province. According to the phylogenetic tree, the new species, *A. kunmingensis*, forms a separate branch in *Amphichorda* and is sister to *A. guana*. However, it differs from *A. guana* by its smaller conidia. Although *A. kunmingensis*, *A. excrementa* and *A. yunnanensis* were all collected from Yunnan, their morphology was quite different (see Table 2). *Amphichorda kunmingensis* differs from *A. excrementa* in its usually curved and longer phialides (6.1–17.5 × 1.4–2.9 μm vs. 4.1–13.9 × 1.3–2.1 μm) and larger conidia (2.3–4.2 × 1.6–3.0 μm vs. 1.7–3.0 × 1.2–2.5 μm). *Amphichorda kunmingensis* differs from *A. yunnanensis* in the shape of its phialides and narrower conidia.

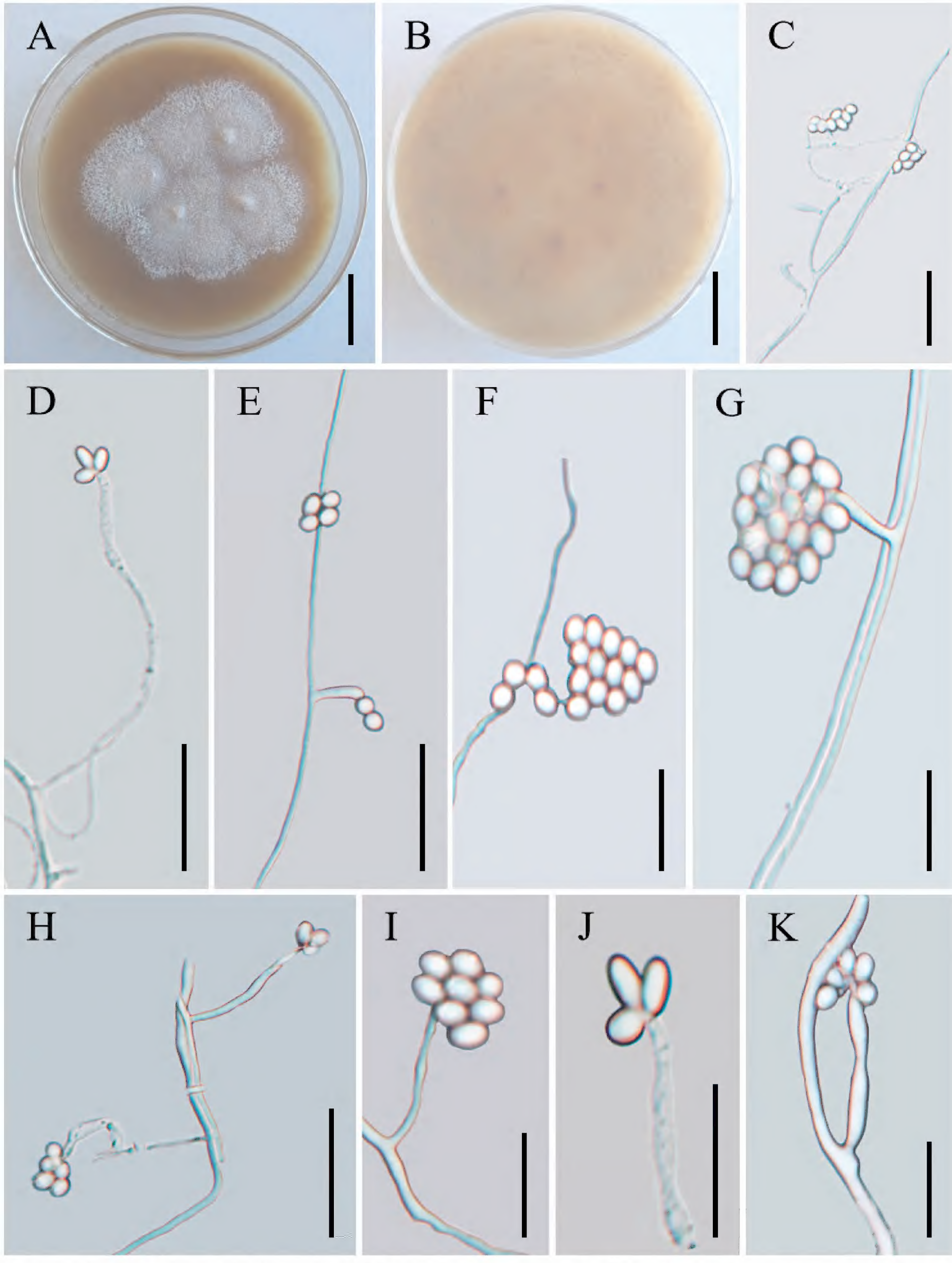

Figure 4. Morphology of *Amphichorda kunmingensis* **A, B** colony character on PDA medium after 30 d (**A** obverse **B** reverse) **C–K** conidiogenous cells and conidia. Scale bars: 2 cm (**A, B**); 20 μm (**C–E, H**); 10 μm (**F–G, I–K**).

Discussion

The phylogenetic analyses, based on the five-gene (nr*SSU*, nr*LSU*, *tef-1α*, *rpb1* and *rpb2*) sequence and ITS data were conducted and *Amphichorda excrementa* and *A. kunmingensis* were introduced. The morphological characteristics of the new species are similar to those of other *Amphichorda* species. Its conidiophores straight or slightly curved; phialides solitary, simple whorls or several whorls, straight or irregularly bent, usually curved, tapering abruptly towards the apex; conidia solitary or clumped, one-celled, shape variable (Table 2). They were similar to those of *Beauveria* and all species of *Amphichorda* do not have the elongate conidiogenous cells with apical denticulate rachis that are characteristic of *Beauveria*.

Table 2. Geographical location, hosts/substrates and asexual morphology of *Amphichorda*.

Species	Country	Host/Substrate	Conidiophores	Phialides (μm)	Conidia (μm)	References
Amphichorda cavernicola	China	Bird faeces; soil; plant debris; animal faeces; bat guano	Cylindrical, straight or slightly curved, occasionally branched	Fusiform or ellipsoidal, straight or irregularly bent, 4.5–8.0 × 2.0–3.0	Broadly ellipsoidal to subglobose, 2.5–4.0 × 2.0–3.5	Zhang et al. (2021)
A. coprophila	Canada; England	Chipmunk, rabbit and porcupine dung	Straight or flexuous, unbranched, bearing lateral or terminal conidiogenous cells, arranged singly or in whorls	Flask-shaped, usually with a strongly bent neck, 6–10 × 2–2.5	Subglobose to somewhat ellipsoidal, 3.5–5.5 × 2–3	Guerra-Mateo et al. (2023)
A. excrementa	**China**	Animal faeces	**Cylindrical, straight or slightly curved, occasionally branched**	**Occasionally solitary, mostly in whorls of 2–3, basal portion cylindrical or flask-shaped, usually curved, 4.1–13.9 × 1.3–2.1**	**Globose to elliptical 1.7–3.0 × 1.2–2.5**	**In this study**
A. felina	Britain, Germany, Argentina, France	Pupa of *Anaitis efformata*; rabbit dung; mouldy leaves; porcupine dung; cat dung	Straight	Solitarily or in small groups, consisting of a swollen, flask-shaped or curved, occasionally elongate basal part, 1.5–8.5 × 1.8–2.9	Subglobose, ellipsoidal or ovoidal, sometimes with a pointed base, 2.5–4.7 × 2–3.5	De Hoog (1972); **In this study**
A. guana	China	Bat guano	Straight or slightly curved	Fusiform or ellipsoidal, straight or irregularly bent, 7–10 × 2–3	Broadly ellipsoid to subglobose, 4.5–5.5 × 3.5–5	Zhang et al. (2017)
A. kunmingensis	**China**	**Animal faeces**	**-**	**Solitary, occasionally in simple whorls, basal portion cylindrical or fusiform, straight or irregularly bent, 6.1–17.5 × 1.4–2.9**	**Globose to elliptical 2.3–4.2 × 1.6–3.0**	**In this study**
A. littoralis	Spain	Sediments; fragment of floating rubber tire	Straight or flexuous, commonly unbranched, bearing lateral or terminal conidiogenous cells, arranged singly or in whorls of 2–4	Flask-shaped, usually with a strongly bent neck, 6–10 (–11.5) × 1.5–2	Subglobose, 3–4 × 2.5–3	Guerra-Mateo et al. (2023)
A. monjolensis	Brazil	on PDA plate consumed by an insect	Cylindrical, bearing one or more conidiogenous cells, straight or slightly bent, solitary or synnematous, sometimes branched	Flask-shaped, straight or irregularly bent, 3.1–6.1 × 2.7–5.1	Holoblastic, 2.8–3.7×1.8–2.9	Leão et al. (2024)
A. yunnanensis	China	Wing surfaces of *Rhinolophus*	Cylindrical, straight or slightly curved, branched	Monoblastic to polyblastic, ampulliform to flask-shaped, 4–12 × 1–4	Globose to oval, slightly ellipsoid, 2–5 × 2–4	Liu et al. (2023)

The species of *Amphichorda* has an extremely wide distribution, including Argentina, Canada, China, France, Germany, Great Britain, Spain (Table 2). Amongst the *Amphichorda* species, *A. felina*, *A. cavernicola*, *A. guana* and *A. monjolensis* were found in caves, especially *A. felina*, which was widely distributed in caves (Vanderwolf et al. 2013; Zhang et al. 2017, 2021; Vanderwolf et al. 2018; Leão et al. 2024). *Amphichorda littoralis* was found in Mediterranean coast sediments at 20 m depth (Guerra-Mateo et al. 2023). In contrast to the particular ecology of caves and the sea, *A. coprophila* was isolated from rabbit, chipmunk and porcupine dung and *A. yunnanensis* was isolated from the wing surfaces of *Rhinolophus affinis* (Guerra-Mateo et al. 2023; Liu et al. 2023). *Amphichorda excrementa* and *A. kunmingensis* were isolated from animal faeces in the Park. The substrates of *Amphichorda* were complex and varied, being mainly animal faeces, i.e. bird, cat, bat, chipmunk, rabbit and porcupine dung, but they have also been isolated in the pupa of *Anaitis effor-mata*, mouldy leaves, plant debris, sediments, fragments of floating rubber tyres, wing surfaces of *Rhinolophus* and soil. Most species of the genus *Amphichorda* have been isolated on animal faeces and are quite unique to their parasitic environments. This is unique to the biological characteristics and ecological habits for the genus *Amphichorda*.

Coprophilous fungi, particularly coprophilous ascomycetes, will be a rich source of antibiotics and other biologically important secondary metabolites (Bills et al. 2013). Species of the genus *Amphichorda* tend to have special physiological and metabolic characteristics due to the uniqueness of their growth environment. Additionally, some of their species have been reported to have high application value, such as *A. felina*, which was a well-known producer of insecticidal cyclodepsipeptide and cyclosporin C (Langenfeld et al. 2011; Chung et al. 2013; Xu et al. 2018). Furthermore, the study by Liang et al. (2021) successfully established a genetic transformation system in *A. guana* strain LC5815, which facilitated the development of bioactive secondary metabolites in fungi. Two new species of the genus *Amphichorda*, described in the present study, were isolated from animal faeces and may have good potential for natural product research.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Natural Science Foundation of China (No. 31870017).

Author contributions

Data curation: QYD. Investigation: YW. Visualization: RL, YLL. Writing - original draft: ZQW. Writing - review and editing: JZ, HY.

Author ORCIDs

Zhi-Qin Wang https://orcid.org/0000-0001-9022-3635
Hong Yu https://orcid.org/0000-0002-2149-5714

Data availability

All of the data that support the findings of this study are available in the main text.

References

Araújo JPM, Lebert BM, Vermeulen S, Brachmann A, Ohm RA, Evans HC, de Bekker C (2022) Masters of the manipulator: Two new hypocrealean genera, *Niveomyces* (Cordycipitaceae) and *Torrubiellomyces* (Ophiocordycipitaceae), parasitic on the zombie ant fungus *Ophiocordyceps camponoti-floridani*. Persoonia 49(1): 171–194. https://doi.org/10.3767/persoonia.2022.49.05

Belyagoubi L, Belyagoubi-Benhammou N, Jurado V, Dupont J, Lacoste S, Djebbah F, Ounadjela FZ, Benaissa S, Habi S, Abdelouahid DE, Saiz-Jimenez C (2018) Antimicrobial activities of culturable microorganisms (Actinomycetes and Fungi) isolated from Chaabe Cave, Algeria. International Journal of Speleology 47(2): 189–199. https://doi.org/10.5038/1827-806X.47.2.2148

Bills GF, Gloer JB, An Z (2013) Coprophilous fungi: Antibiotic discovery and functions in an underexplored arena of microbial defensive mutualism. Current Opinion in Microbiology 16(5): 549–565. https://doi.org/10.1016/j.mib.2013.08.001

Bischof JF, Rehner SA, Humber RA (2006) *Metarhizium frigidum* sp. nov.: A cryptic species of *M. anisopliae* and a member of the *M. favoviride* Complex. Mycologia 98(5): 737–745. https://doi.org/10.1080/15572536.2006.11832645

Carmichael JW, Kendrick WB, Conners IL, Sigler L (Eds) (1980) Genera of Hyphomycetes. University of Alberta Press.

Chung YM, El-Shazly M, Chuang DW, Hwang TL, Asai T, Oshima Y, Ashour ML, Wu YC, Chang FR (2013) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, induces the production of anti-inflammatory cyclodepsipeptides from *Beauveria felina*. Journal of Natural Products 76(7): 1260–1266. https://doi.org/10.1021/np400143j

De Hoog GS (1972) The Genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. Studies in Mycology 1: 1–41.

Fries EM (1825) Systema orbis vegetabilis: Primas Lineas Novae Constructionis Periclitatur; E Typographia Academica. Lunde, Norway 1: 170.

Guerra-Mateo D, Gené J, Baulin V, Cano-Lira JF (2023) Phylogeny and Taxonomy of the Genus *Amphichorda* (Bionectriaceae): An Update on *Beauveria*-like Strains and Description of a Novel Species from Marine Sediments. Diversity 15(7): 795. https://doi.org/10.3390/d15070795

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Hou LW, Giraldo A, Groenewald JZ, Rämä T, Summerbell RC, Huang GZ, Cai L, Crous PW (2023) Redisposition of acremonium-like fungi in Hypocreales. Studies in Mycology 105(1): 23–203. https://doi.org/10.3114/sim.2023.105.02

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Lamarck JB (1815) Flore Françoise ou Descriptions Succinctes de Toutes les Plantes Qui Croissent Naturellement en France, Disposées Selon Une Nouvelle Méthode D'Analyse, et Précédées Par un Exposé des Principes Élémentaires de la Botanique. Libraire Chez Desray, Paris, vol. 6, 30 pp. https://doi.org/10.5962/bhl.title.112968

Langenfeld A, Blond A, Gueye S, Herson P, Nay B, Dupont J, Prado S (2011) Insecticidal cyclodepsipeptides from *Beauveria felina*. Journal of Natural Products 74(4): 825–830. https://doi.org/10.1021/np100890n

Leão AF, Condé TO, Dutra YLG, Rosado AWC, Grazziotti PH, Neves SC, Fraga LMS, Kasuya MCM, Pereira OL (2024) *Amphichorda monjolensis* sp. nov., a new fungal species isolated from a Brazilian limestone cave, with an update on acremonium-like species in Bionectriaceae. Brazilian Journal of Microbiology. https://doi.org/10.1007/s42770-024-01289-y

Lendemer JC (2020) Epitypes are forever: Best Practices for an Increasingly Misused Nomenclatural Action. Taxon 69(5): 849–850. https://doi.org/10.1002/tax.12289

Liang M, Li W, Qi D, Chen C, Cai L, Yin B. (2021) Establishment of a genetic transformation system in guanophilic fungus *Amphichorda guana*. Journal of Fungi 7: 138. https://doi.org/10.3390/jof7020138

Liu M, Hodge KT (2005) *Hypocrella zhongdongii* sp. nov., the teleomorph of *Aschersonia incrassata*. Mycological Research 109: 818–824. https://doi.org/10.1016/10.1017/S095375620500290X

Liu XF, Tibpromma S, Hughes AC, Chethana KWT, Wijayawardene NN, Dai DQ, Du TY, Elgorban AM, Stephenson SL, Suwannarach N, Xu JC, Lu L, Xu RF, Maharachchikumbura SSN, Zhao CL, Bhat DJ, Sun YM, Karunarathna SC, Mortimer PE (2023) Culturable mycota on bats in central and southern Yunnan Province, China. Mycosphere 14(1): 497–662. https://doi.org/10.5943/mycosphere/14/1/7

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and efective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98(6): 625–634. https://doi.org/10.1016/S0953-7562(09)80409-7

Rehner SA, Minnis AM, Sung GH, Luangsa-ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103(5): 1055–1073. https://doi.org/10.3852/10-302

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efcient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Sung GH, Hywel-Jones NL, Sung JM, Luangsa-Ard JJ, Shrestha B, Spatafora JW (2007) Phylogenetic classifcation of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57: 5–59. https://doi.org/10.3114/sim.2007.57.01

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Vanderwolf KJ, Malloch D, Mcalpine DF, Forbes GJ (2013) A world review of fungi, yeasts, and slime molds in caves. International Journal of Speleology 42(1): 77–96. https://doi.org/10.5038/1827-806X.42.1.9

Vilgalys R, Hester M (1990) Rapid genetic identifcation and mapping of enzymatically amplifed ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wang YB, Yu H, Dai YD, Wu CK, Zeng WB, Yuan F, Liang ZQ (2015a) *Polycephalomyces agaricus*, a new hyperparasite of *Ophiocordyceps* sp. infecting melolonthid larvae in southwestern China. Mycological Progress 14(9): 70. https://doi.org/10.1007/s11557-015-1090-7

Wang YB, Yu H, Dai YD, Chen ZH, Zeng WB, Yuan F, Liang ZQ (2015b) *Polycephalomyces yunnanensis* (Hypocreales), a new species of *Polycephalomyces* parasitizing *Ophiocordyceps nutans* and stink bugs (Hemipteran adults). Phytotaxa 208: 34–44. https://doi.org/10.11646/phytotaxa.208.1.3

Wang YB, Wang Y, Fan Q, Duan DE, Zhang GD, Dai RQ, Dai YD, Zeng WB, Chen ZH, Li DD, Tang DX, Xu ZH, Sun T, Nguyen TT, Tran NL, Dao VM, Zhang CM, Huang LD, Liu YJ, Zhang XM, Yang DR, Sanjuan T, Liu XZ, Yang ZL, Yu H (2020) Multigene phylogeny of the family Cordycipitaceae (Hypocreales): New taxa and the new systematic position of the Chinese cordycipitoid fungus *Paecilomyces hepiali*. Fungal Diversity 103(1): 1–46. https://doi.org/10.1007/s13225-020-00457-3

Wang ZQ, Wang Y, Dong QY, Fan Q, Dao VM, Yu H (2022) Morphological and Phylogenetic Characterization Reveals Five New Species of *Samsoniella* (Cordycipitaceae, Hypocreales). Journal of Fungi (Basel, Switzerland) 8(7): 747. https://doi.org/10.3390/jof8070747

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplifcation and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Xu L, Li Y, Biggins JB, Bowman BR, Verdine GL, Gloer JB, Alspaugh JA, Bills GF (2018) Identifcation of cyclosporin C from *Amphichorda felina* using a *Cryptococcus neoformans* differential temperature sensitivity assay. Applied Microbiology and Biotechnology 102(5): 2337–2350. https://doi.org/10.1007/s00253-018-8792-0

Zhang ZF, Liu F, Zhou X, Liu XZ, Liu SJ, Cai L (2017) Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. Persoonia 39(1): 1–31. https://doi.org/10.3767/persoonia.2017.39.01

Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Zhang ZF, Zhou SY, Eurwilaichitr L, Ingsriswang S, Raza M, Chen Q, Zhao P, Liu F, Cai L (2021) Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Diversity 106(1): 29–136. https://doi.org/10.1007/s13225-020-00453-7